Influence of Temperature on Embryogenesis in Meloidogyne javanica

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Abstract: The optimum temperature for embryogenesis in *Meloidogyne javanica* lies between 25 and 30 C. Embryogenesis is slightly more rapid at 30 C (9-10 days), but more eggs complete development at 25 C (11-13 days). At temperatures of 25, 27.5, and 30 C, embryogenesis is about twice as rapid as at 20 C (23-25 days), and about four times as rapid as at 15 C (46-48 days). Time-lapse studies showed that the thermal optimum is similar throughout the different stages of embryonic development. *Key words:* embryo, larva, ciné time-lapse.

The first report on the influence of temperature on egg development in root-knot nematodes was by Tyler (6) in 1933. She found that embryogenesis of eggs in hanging drop cultures was completed in 9 days at 27 C, whereas it took 31 days at 16.5 C. Bird and Wallace (3) showed that over a period of 6 days, the thermal optimum for the hatch of eggs of *Meloidogyne javanica* (Treub) Chitwood was in the region of 30 C. Subsequently, Wallace (7) reported that the thermal optimum for embryogenesis is about 15 C, whereas that for eclosion (emergence of larva from egg or what is commonly known as hatching) is 30 C.

To determine whether different stages of embryogenesis of *M. javanica* have different thermal optima, I studied its embryogenesis at different temperatures using time-lapse ciné photomicrography to record every phase of development. Detailed descriptions of the morphological changes occurring during embryogenesis are not given here, as they did not deviate from descriptions already published (2, 4, 5).

MATERIALS AND METHODS

Egg masses of *M. javanica* containing a relatively large proportion of eggs in their earliest stages of development (4-6 weeks from time of infection in glass house) were dissected from the roots of tomato plants. The egg masses were vibrated in a hypochlorite solution

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(0.5% available chlorine) for 1 min and washed in sterile distilled water, and the eggs collected by centrifugation. Exposure to the hypochlorite solution did not exceed 3 min. The eggs were further surface sterilized by treatment with 0.5% Hibitane® (chlorhexidine gluconate) for 10 min, followed by three washes in sterile distilled water. It was found that this further reduces bacterial contamination without affecting the rate of embryonic development.

Eggs in the 2-celled stage were selected carefully and placed either in 0.5 ml of distilled water in watch glasses sealed with petroleum jelly for counting experiments, or in sitting drop slides (2) for photomicrography. All manipulations were done under aseptic conditions. Counting experiments consisted of counting the number of eggs which had become first stage larvae or had hatched at a particular time for each temperature range; 50 ± 3 eggs were used per watch glass, and 6-8 eggs were used in the time-lapse studies in each sitting drop slide.

Ciné time-lapse photographs were taken on 35-mm film at 30-min intervals using a Zeiss micro ciné camera in conjunction with a temperature-controlled box (Fig. 1) with temperatures selected at 20, 25, 27.5, and 30 C (all ± 0.25 C). For the counting experiments, the watch glasses were kept in incubators set at 15, 20, 25, 30, and 35 C, respectively (all ± 0.5 C).

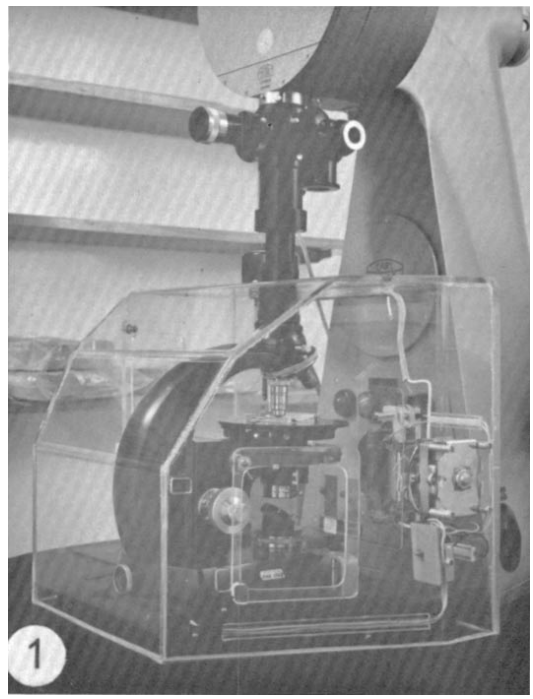

FIG. 1. The apparatus used for ciné time-lapse photomicrography.

RESULTS

In studies involving comparison of rates of embryogenesis, it is most important to select similar early stages so that development is synchronous. The results of this type of selection are shown in Fig. 2, where the development of a batch of 7 eggs in a sitting drop slide is followed at 27.5 C. In this particular case, 5 of the 7 eggs completed their development, and it was only during the development of tadpole larvae to first stage larvae (Fig. 2E), which takes place within a few hours, that any differences could be detected in their otherwise synchronous rates of development.

The rate of embryogenesis of *M. javanica* at different temperatures varied considerably (Fig. 3). These photographs of eggs selected for uniformity were taken over a 9-day period, during which time egg development at 30 C had reached the stage of shell plasticity (Fig. 3H) which is known to precede hatching (1).

Rates of development at 25 and 30 C were similar, and were greater than at 20 and 15 C. Thus at 20 C, newly formed, first stage larvae were observed in 9 days (Fig. 3, 20 C, H), whereas at 30 C they were observed at 4.75 days (Fig. 3, 30 C, E). Embryogenesis at 20 C proceeded at about half the rate as at 30 C. The rate of development at 15 C was much slower, and embryos in the early gastrula stage were observed at 9 days (Fig. 3, 15 C, H), whereas at 20 C this stage was observed at 4.75 days (Fig. 3, 20 C, E), and at 30 C it was observed between 1.25 and 3.75 days (Figs. 3, 30 C, C, D). Thus, the rate of embryogenesis at 15 C appeared to be about 4-5 times slower than at 30 C.

Even though embryogenesis in some cases took several weeks (e.g., at 20 C), the use of time-lapse ciné equipment ensured that even changes which occurred comparatively rapidly were recorded. Thus, the obvious and rapid morphological changes which occurred during the formation of a first stage larva from the tadpole stage (Fig. 4) took about 7 hr at 20 C, whereas at 30 C it took only about 3.5 hr.

Stages of embryogenesis of large batches of

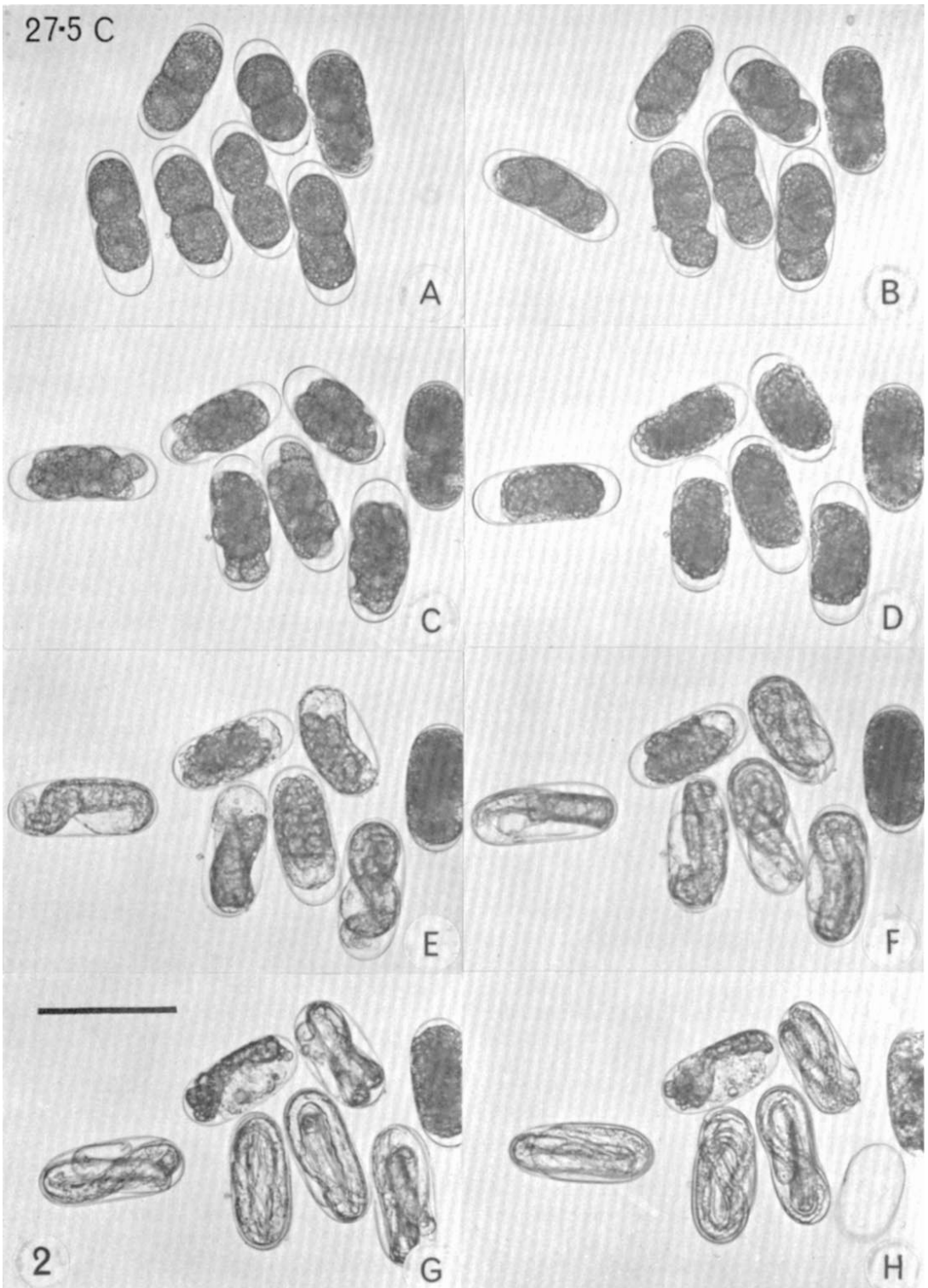

FIG. 2. Synchronous embryogenesis of *Meloidogyne javanica* at 27.5 C. Bar = 100 μm. A = 2-cell stage, start; B = 4-cell stage, 0.5 days; C = multicell stage, 1 day; D = gastrula stage, 3 days; E = tadpole stages, 4 days; F = first-stage larvae, 4.5 days; G = second-stage larvae, 8 days; H = first egg hatched, 11 days.

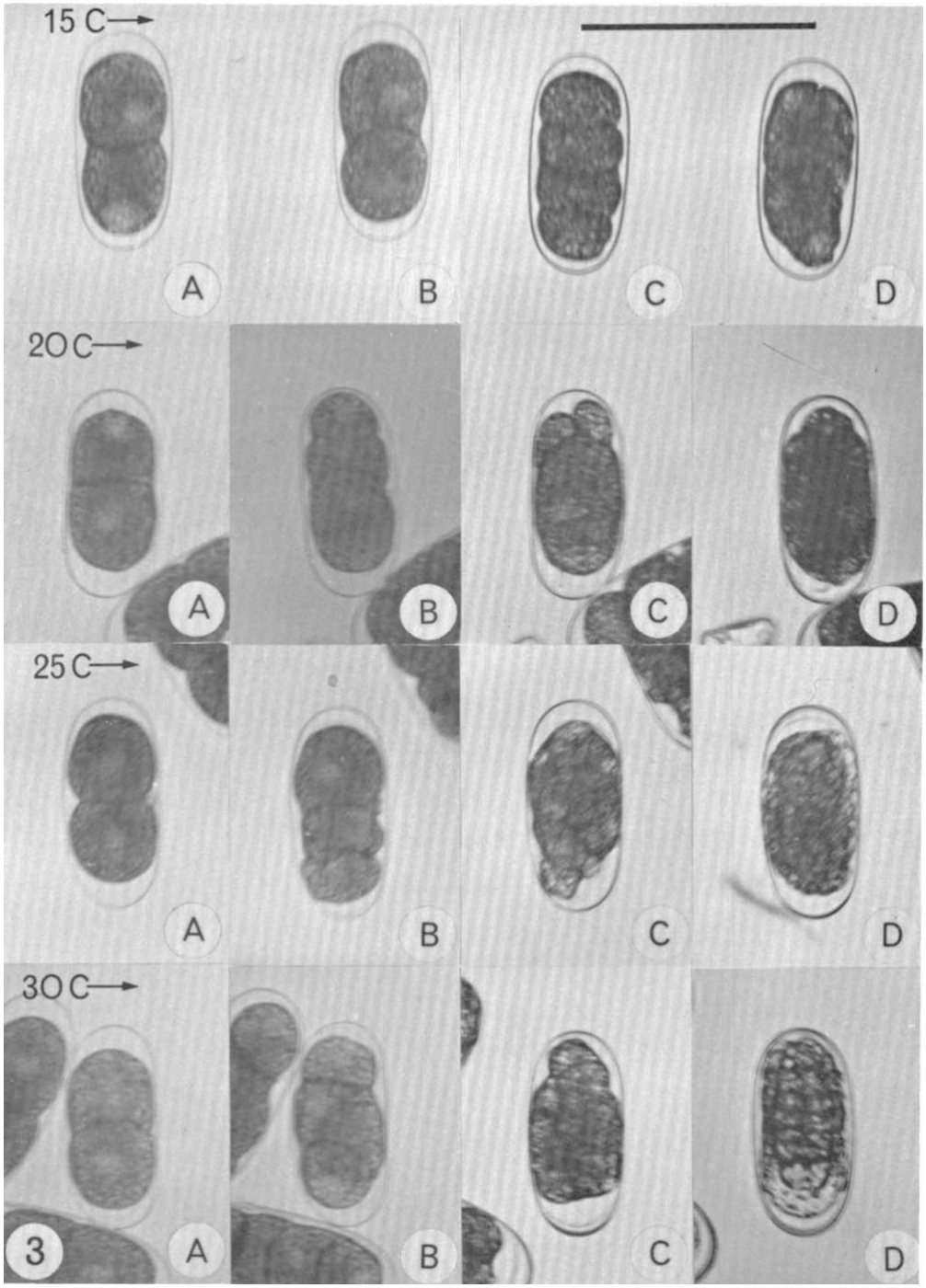

FIG. 3A. Comparative embryogenesis of *Meloidogyne javanica* at 15, 20, 25, and 30 C. Bar = 100 μm. A = 0.25 days; B = 0.75 days; C = 1.25 days; D = 3.75 days.

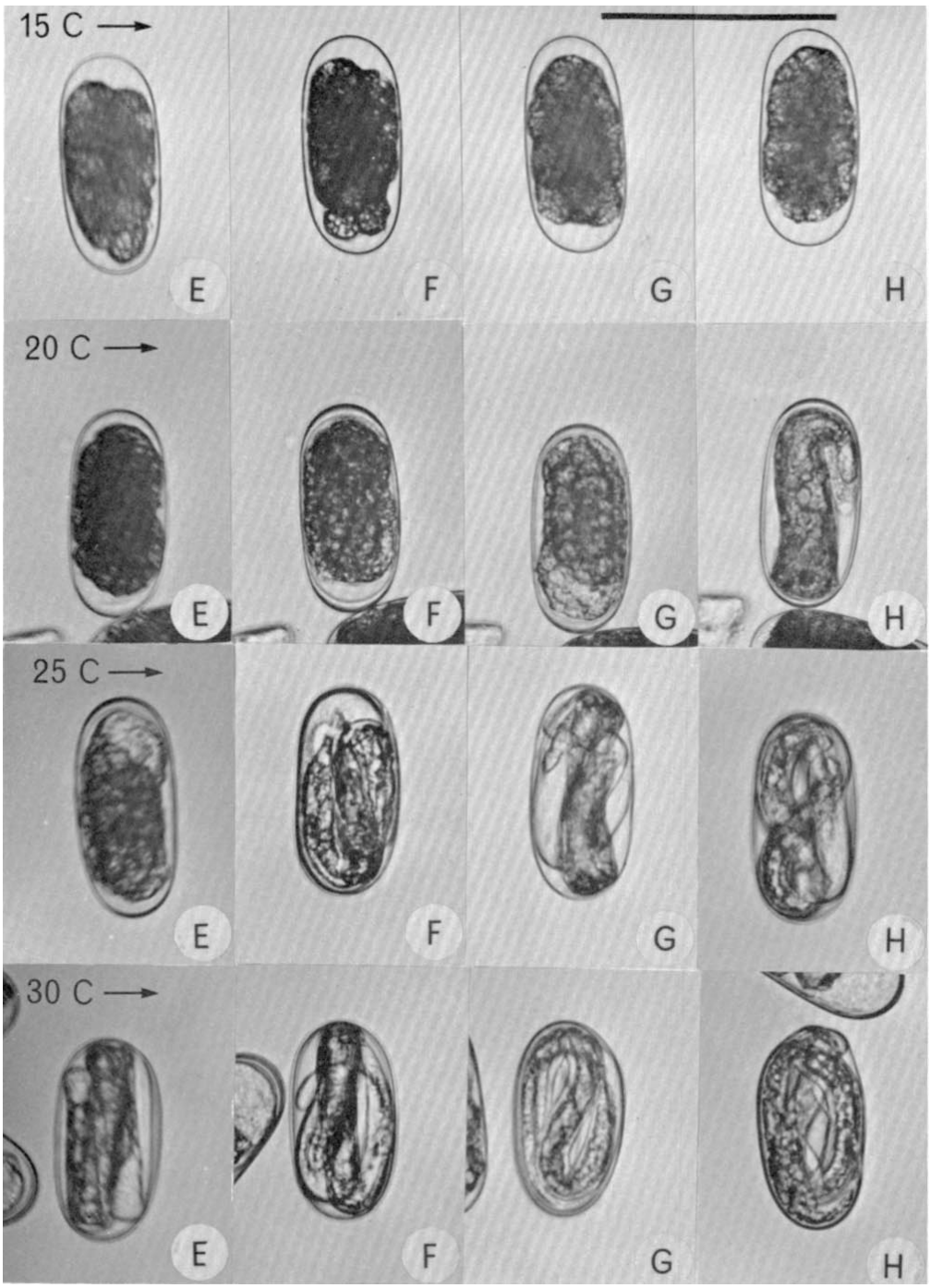

FIG. 3B. Comparative embryogenesis of *Meloidogyne javanica* at 15, 20, 25, and 30 C. Bar = 100 μm. E = 4.75 days; F = 7 days; G = 8 days; H = 9 days.

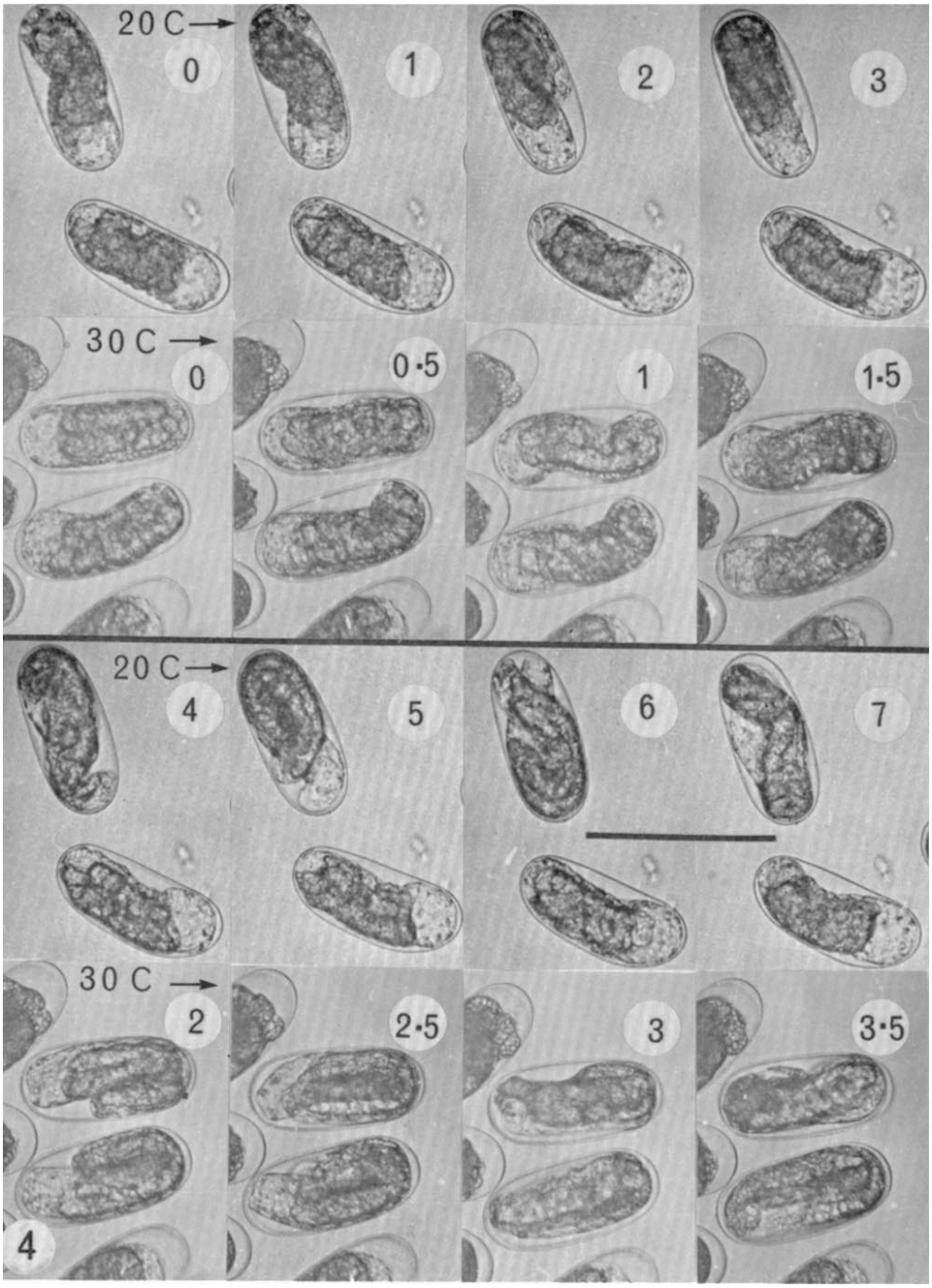

FIG. 4. Comparative embryogenesis of *Meloidogyne javanica* at 20 and 30 C of the development of the tadpole stage to the first larva using ciné time-lapse of 1 frame every 0.5 hr. Bar = 100 μm. 20 C = every second frame; 30 C = every frame.

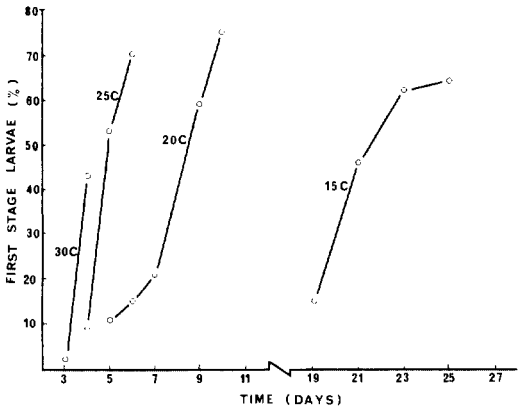

FIG. 5. Embryogenesis of eggs of *Meloidogyne javanica* from the two-cell stage to the first larval stage at 15, 20, 25, and 30 C. The last point on each curve represents maximum number of first stage larvae.

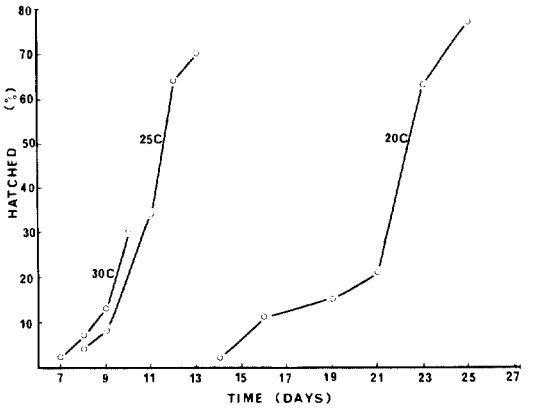

FIG. 6. Embryogenesis of eggs of *Meloidogyne javanica* from the first larval stage through to hatching at 20, 25, and 30 C. The last point on each curve represents maximum number of hatched eggs.

eggs during development in sealed watch glasses were counted at temperatures of 15, 20, 25, and 30 C.

Development up to the first larval stage took 3-4 days at 30 C, but only about 40% of the eggs reached this stage of development (Fig. 5). At 25 C, 4-6 days were required for development to the first larval stage, but about 70% of the eggs developed. At 20 C, development to the first larval stage took about 7-10 days, and 75% of the eggs reached this stage. At 15 C, development to the first larval stage took 19-25 days, and just over 60% of the eggs reached this stage.

Development through the two larval stages to hatching (Fig. 6) took about 9-10 days at 30 C with only about 30% hatching; at 25 C it took 10-13 days with 70% hatching; and at 20 C it took 21-25 days with nearly 80% hatching. The curve for 15 C is not included in this graph because no larvae hatched by 27 days and, in fact, hatching took 44-51 days and only 50% hatched. Embryogenesis was never completed at 35 C, and development usually ceased within 4 days.

DISCUSSION

The results described above indicate that the thermal optimum for embryogenesis in *M. javanica* lies between 25 and 30 C, and that this appears to be the optimum temperature for development in all stages of embryogenesis.

The mortality of embryos at 30 C is twice as great as at 25 C, but the difference in embryo mortality between 25 and 20 C is slight and is offset by the much slower rate of development at 20 C.

At 15 C, the rate of embryogenesis is extremely slow, and these results contradict the hypothesis (7) that this temperature is close to the thermal optimum for embryogenesis in *M. javanica* or that the thermal optimum for embryogenesis differs very much, if at all, from that for hatching or eclosion. It is difficult to explain these conflicting results since similar materials and methods were used. They may be due partly to a greater depth of water in the watch glasses, coupled with the possibility of contamination with aerobic bacteria having a thermal optimum of 25-30 C in Wallace's experiments. These factors could induce an oxygen deficiency which could increase with rise in temperature. However, these ideas could account only partly for the differences that have been described as similar results were obtained without the use of Hibitane.

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